Other Aspects of Metabolic Monitoring in Critically Ill Patients

Frank N. Konstantinides,1 Peggy L. Kaproth,1 and Frank B. Cerra2

Cardiopulmonary monitoring remains the mainstay of intensive-care unit utilization of clinical chemistry resources. Its focus has been on the restoration and maintenance of oxygen transport. Metabolic monitoring, a relatively new area of application for clinical chemistry technology, focuses on cell metabolism and on cell–cell interaction as a mechanism of metabolic regulation. This latter application of monitoring is developing as a result of a better understanding of the pathogenesis of organ dysfunction and disease processes in intensive-care unit patients. Some of the clinical chemistry technologies used include analyses for amino acids and polyunsaturated fatty acids, measurement of cytokine concentration and activity, nutritional assessment and monitoring, and sensitive monitors of liver function, and assessment of altered immunity in critically ill patients. Use of these technologies, along with specific support measures, offers new avenues for decreasing infectious complications and reducing mortality and morbidity of patients in intensive-care units.

Longitudinal profiles of plasma metabolites in patients after general surgery, in trauma, and during systemic sepsis were conducted in the 1960s and 1970s (1–9). Although an increasing severity of injury correlated with an increasing magnitude of response, "typical" response patterns for each clinical prototype could be identified (1, 10) (Figure 1, Table 1).

These studies indicated that the injury of trauma, surgery, and sepsis induced a systemic neurohumoral-mediated metabolic response that was reflected in the plasma metabolic profile and in the urinary metabolites. Several of these measurements correlated with outcome; many led to a better understanding of the pathogenesis of this response and its sequelae of multiple organ failure. Other measurements provided clues that eventually led to new treatment regimens currently in clinical testing or that have become commonplace in clinical practice.

One of the best examples of this process occurred in the area of nutritional support in the intensive-care unit (ICU).3 The response to trauma and sepsis includes an accelerated synthetic rate of proteins essential for maintenance of wound healing and preservation of organ function (10–15). Muscle proteolysis supplies these amino acids that serve as substrate for protein synthesis, as well as oxidative fuels for energy production. These alterations are reflected in the plasma amino acid profiles; increased urinary nitrogen excretion and negative nitrogen balance; glucose intolerance with insulin resistance; decreased oxidation of glucose; reduced triglyceride clearance, particularly for long-chain fatty acids; and altered patterns of polyunsaturated fatty acids (PUFA) in plasma (16).

These monitored alterations were correlated with changes in organ function and in outcome. Some of these alterations included wound failure, wound infection, an increased incidence of nosocomial infections, immunosuppression, decubitus ulcers (bedsores), gastrointestinal bleeding, encephalopathy, and single or multiple organ failure.

It became apparent that requirements for protein, fat, and carbohydrate were quite different in the critically ill patient. With the plasma amino acid profiles as guides, and data on urinary nitrogen excretion, new amino acid formulations were developed and tested and are now in clinical use in the ICU. Continued research has now identified nutrients that can alter targeted organ function, such as T lymphocyte suppression and macrophage hyperactivity. These therapies are now in clinical testing.

Here we briefly review some of these observations and their current clinical application. We also present forthcoming metabolic monitors, with some discussion of their rationale and projected clinical utility.

Amino Acids in Plasma

Spackman et al. (17) first introduced commercially automated data acquisition into amino acid analysis in 1958. In part for this work, they received a Nobel Prize in chemistry. Since that time, technical improvements in the instrumentation for amino acid analysis, along with an improved understanding of amino acid metabolism in ICU patients, have allowed its transition from a research tool to a useful analysis in clinical nutrition practice.

Amino acid analysis has had two general applications to physiological fluids. One, the detection and management of inborn errors of metabolism, will not be discussed in this paper. The other application, the subject of this section, is the evaluation of the metabolic response to injury and the development and monitoring of nutritional support regimens in the critically ill patient.

The earliest evaluation of amino acid technology was in patients with cirrhosis and encephalopathy. The low concentrations of branched-chain amino acids in plasma and the increases of aromatic and sulfur-containing amino acids were recognized as characteristic of the disease process, as was the increased concentration of glutamine in the spinal fluid. An amino acid formula was designed to normalize the profile of amino acids in plasma when given in adequate doses. Clinical studies with this approach have demonstrated both improved recovery from encephalopathy and reduced mortality (18) (Figure 2).

When glucose-based nutrition was applied to patients in sepsis and hypermetabolism, several adverse consequences resulted: increased oxygen consumption and carbon dioxide production, increased catecholamine release, fatty liver, pulmonary failure, and poor support of protein synthesis

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1 Surgical Metabolic Research Facility, St. Paul–Ramsey Medical Center, 640 Jackson St., St. Paul, MN 55101.
2 Department of Surgery, University of Minnesota Hospital and Clinics, Minneapolis, MN, and St. Paul–Ramsey Medical Center, St. Paul, MN.
3 Nonstandard abbreviations: ICU, intensive-care unit; PUFA, polyunsaturated fatty acids; MOFS, multiple organ failure syndrome; TUN, total urea nitrogen; UUN, urinary urea nitrogen; TNF, tumor necrosis factor; and IL, interleukin.

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(19). Analysis of energy expenditure revealed that reduced caloric loads and reduced glucose loads were necessary to avoid some of these complications in most patients. Amino acids were found to be the most effective anticatabolic support, primarily because they led to an increase in protein synthesis rate. Analysis of amino acid profiles and kinetics revealed an increased use of the branched-chain amino acids for energy, primarily in skeletal muscle, and an increased mobilization of amino acids from muscle stores and transport to active sites of protein synthesis and gluconeogenesis such as liver. Because of these findings and the ureagenic potential of standard amino acid formulas, new amino acid formulations were developed that were increased in the concentrations of branched-chain amino acids and decreased in the glucogenic and aromatic amino acids. Clinical studies with these new formulas have indicated that they are more efficient at promoting nitrogen balance while supporting hepatic protein synthesis and minimizing urea production (11).

The low concentrations of glutamine in plasma from these metabolically stressed patients led to a series of investigations that focused on glutamine metabolism in septic states. The low concentration appears to be related to glutamine's role as a gut energy source and a trophic factor for the gut mucosa (20). Clinical studies are now underway to verify the potential clinical utility of this amino acid.

In addition, arginine has been recognized as a modulator of lymphocyte proliferative response and as one of the products of the activated macrophage that can modulate hepatocellular function (21). Proposed effects of arginine will be discussed in greater detail later in this chapter.

Hepatic production of protein is closely related to the liver's clearance of amino acids. Experiences gained during liver transplantation led to this insight. In this setting the concentration of total free amino acids in plasma increases fivefold over baseline during the anhepatic phase, then decreases to baseline values or below after successful allografting and production of acute-phase proteins (22, 23). Consequently, this concept of examining amino acid clearance as a test of liver function was proposed (24). The conceptual framework of amino acid clearance has been concurrently developed by two groups of investigators. Clowes et al. (25) developed the "central plasma clearance rate" of amino acids to measure the flux of amino acids

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**Table 1. Categories of Metabolic Stress**

<table>
<thead>
<tr>
<th>Stress</th>
<th>Lactate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Glucose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Leucine</th>
<th>Proline</th>
<th>Phenylalanine</th>
<th>Nitrogen, g/day&lt;sup&gt;a&lt;/sup&gt;</th>
<th>3-Methylhistidine, μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;1000</td>
<td>5.5 ± 2</td>
<td>120 ± 10</td>
<td>200 ± 20</td>
<td>60 ± 15</td>
<td>&lt;5</td>
<td>&lt;100</td>
</tr>
<tr>
<td>1</td>
<td>1200 ± 200</td>
<td>9.5 ± 1.4</td>
<td>74 ± 12</td>
<td>213 ± 4</td>
<td>74 ± 8</td>
<td>5–10</td>
<td>130 ± 20</td>
</tr>
<tr>
<td>2</td>
<td>1200 ± 200</td>
<td>9.5 ± 1.4</td>
<td>74 ± 12</td>
<td>13 ± 40</td>
<td>74 ± 8</td>
<td>10–15</td>
<td>200 ± 20</td>
</tr>
<tr>
<td>3</td>
<td>3000 ± 500</td>
<td>16 ± 1.6</td>
<td>180 ± 30</td>
<td>300 ± 50</td>
<td>124 ± 17</td>
<td>&gt;15</td>
<td>450 ± 50</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results shown are mean ± SD.

<sup>b</sup> In the presence of lactate/pyruvate ratio <20.

<sup>c</sup> In the absence of steroid therapy, diabetes mellitus, or pancreatitis.

<sup>d</sup> Range (by definition).
between peripheral tissues (primarily skeletal muscle) and the visceral organs (liver). During exogenous infusion of amino acids, they measured blood flow while simultaneously quantifying arterial and femoral venous samples in amino acid analysis. The rate of amino acids extracted by the liver was calculated as the quantity of plasma (mL/m² per minute) from which amino acids are cleared. This concept was then applied to patients who were septic or cirrhotic or who had undergone liver transplantation; the measurements were found to have prognostic significance (25, 26).

Konstantinides et al. (27, 28), while developing a canine model of global hepatic ischemia, developed a bolus technique for measuring clearance of amino acids from plasma. After taking a baseline blood sample, they delivered a bolus injection of amino acids (0.5 mL/kg of body wt.) via a venous line and took blood samples 2, 5, 10, and 20 min post-injection. Using a fluorescent label (o-phthalaldehyde) that binds to an amino nitrogen, they could measure the total free amino acid content in plasma with a centrifugal analyzer and determine amino acid clearance (28). This clearance correlated closely with the hepatic energy state and had prognostic significance in liver transplantation as an index of allograft viability and function (22, 23, 29–31) (Figure 3).

Amino acid analysis is also used in assessing hepatic function and as a prognostic index in multiple organ failure syndrome (MOFS). This syndrome can develop after a variety of insults, including tissue injury or ischemia, severe hemorrhage, sepsis, and severe inflammation, as in pancreatitis (19, 32). It involves a complex interaction between the central nervous system, the endocrine system, and cell-cell communication mechanisms. Sequential organ involvement begins in the lung, then proceeds to the liver, kidney, and eventual collapse of the entire metabolic system. MOFS remains the cause of most mortality and prolonged ICU stays in surgical patients (33).

Current severity indexing systems are not applicable in these clinical settings. Rather, metabolic variables must be considered because the process is one of metabolic failure that is poorly reflected in the initial physiology but becomes clinically manifest 7–10 days after injury. In settings where the serum bilirubin may not reflect this injury, such as hemolysis or hematoma resolution, the concentration of phenylalanine in plasma appears to reflect the hepatocellular function and to be of prognostic significance. This observation presumably reflects both the increased release of this amino acid from muscle and the reduced hepatic clearance as the liver function deteriorates during MOFS.

**Acute-Phase Proteins and Nitrogen Balance**

Visceral proteins, including albumin, prealbumin, transferrin, and retinol-binding protein were first used in epidemiological surveys to evaluate nutritional states of populations. Later these proteins were used to evaluate the need for and response to specialized nutritional therapy (34–36). Although visceral proteins have been correlated with morbidity and mortality, they appear to have poor sensitivity and specificity as markers for nutritional status and as monitors of nutritional therapy in individual ICU patients with hypermetabolism and MOFS (37, 38).

Factors other than nutrient availability can influence the concentrations of visceral protein in serum. The most significant of these factors are hormones and mediators that regulate protein synthesis in hepatocytes. Tissue injury seems to stimulate the production of cytokines by macrophages, which in turn regulate hepatocytes to increase production of acute-phase reactants while decreasing the production of other proteins.

Although measurement of serum proteins is easier than a nitrogen balance study, nitrogen balance remains the best bedside tool for assessment of protein utilization and overall nitrogen economy, even in the critically ill patient.

A negative nitrogen balance indicates net protein catabolism. A positive nitrogen balance indicates nitrogen retention and, when adjusted for changes in weight and blood urea nitrogen, generally correlates with net protein synthesis. During nutritional support, the goal is to achieve a nitrogen balance in the range of +2 to +4 g/day (39, 40). Achieving this endpoint indicates that the greatest possible stimulation of protein synthesis with amino acid loading has occurred. In contrast to starvation, where nitrogen equilibrium or positive balance indicates a conservative state, the stressed ICU patient continues in a state of net catabolism and loss of skeletal muscle mass. Dominant
sites of protein synthesis reside in other locations such as liver, wounds, and sites of active inflammation.

Accurate determination of nitrogen balance is important in prescribing the nutritional treatment regimen to be used, as well as to monitor the regimen's efficacy. The ideal determination of nitrogen output requires measurement of nitrogen content from all body fluids excreted. The nonurinary losses, however, are quite predictable in the absence of diarrhea and prolonged hyperthermia. Nitrogen losses from nasogastric drainage in most adults are generally of little clinical significance. Therefore, urine is most commonly collected for determining nitrogen loss, with a stress-adjusted amount for unmeasurable loss added to it, usually 2–4 g/day. Nitrogen input is calculated from the grams of protein delivered, and nitrogen balance is calculated by subtracting nitrogen output from the nitrogen intake.

Several clinical studies indicate that under steady-state conditions of stress and with constant nutritional administration, the use of partial urine collections is reasonably accurate for clinical use (4, 6, or 12-h collection extrapolated to 24 h). Full 24-h collections provide more accurate measurements of nitrogen losses (41). Konstantinides et al. (42) reported that, in the absence of nitrogen-splitting bacteria, the need for icing during 24-h urine collections was not necessary; for research purposes, urine samples can be frozen and batch-analyzed for urea nitrogen or total nitrogen as well as creatinine without major variance in the values (43).

When calculating nitrogen balance by using urinary urea nitrogen (UUN) as the estimate of nitrogen loss, UUN is commonly corrected for the nonurea component by increasing the UUN measured by 20%. Another factor may be added for the relatively constant nitrogen loss through stool and skin. All measured and calculated components would then represent the total nitrogen loss (44). Investigators have shown that UUN represents approximately 80% of the total urinary nitrogen (TUN) in healthy, non-stressed surgical patients (45, 46). However, when applied to a clinical population with various degrees of stress during the course of an illness or during various disease states, this calculation from UUN does not accurately reflect the TUN (44–47).

In a recent study of general surgical patients that was designed to evaluate the accuracy of estimating TUN output from the measured UUN, paired studies of UUN: TUN were performed on 421 24-h urine collections from general surgical/trauma patients. The variability of UUN as a percentage of TUN ranged from 12% to 100% (48). If these UUN values are used as estimates for TUN in calculating nitrogen balance, variations of up to 12 g/day could result (49). This study also demonstrated that the correction factor of 1.25 for estimating TUN from UUN was not reliable.

Elsewhere we suggested (50) that Pyro-Chemiluminescence™, which has excellent correlation with the classic Kjeldahl technique, can be used for determining nitrogen content in urine, feces, and other biological fluids cheaply, rapidly, and in real-time (51–54). In the absence of clinically useful markers of protein synthesis in these ICU settings, nitrogen balance will probably remain the dominant marker of nutritional outcome.

**Ketone Bodies**

Ketone bodies (acetoacetate and β-hydroxybutyrate) are produced exclusively in the liver by partial oxidation of fatty acids. These ketones can be oxidized for energy metabolism, and are primarily utilized by cardiac and skeletal muscle during glucose deprivation. In nonseptic injury or starvation, increased availability of ketone energy substrate attenuates enhanced catabolism of endogenous protein (55, 56). Although trauma, major surgery, and sepsis result in an increased demand for oxidative substrates, studies have demonstrated depressed concentrations of ketones in fasting plasma under these conditions (57–59).

Vary et al. (60) proposed that increased malonyl-CoA inhibits acetyl-CoA production (ketone body precursor) in sepsis. Failure of hepatic tissue to enhance ketogenesis may be important to the outcome of a septic episode, because survival depends on normal hepatic function (16, 61). Functional failure of the liver is increasingly being recognized as a key factor in the pathogenesis of postoperative MOFS (5, 62).

The reduction of acetoacetate to β-hydroxybutyrate is a key metabolic reaction within liver mitochondria and involves the production of energy-rich ketone bodies. The amount of conversion of acetoacetate to β-hydroxybutyrate is determined by the ratio of NADH to NAD+. Indeed, the relative amounts of the two oxybutyrate produced by the liver are used as an index of the state of reduction of NAD+ in mitochondria. The formation of hydroxybutyrate is done by withdrawing electrons from the mitochondria to make a more reduced substrate. Later oxidation of the compound in peripheral tissue can produce more high-energy phosphates than does oxidation of acetoacetate (63). Inhibition of the electron-transport system may be caused by mitochondrial impairment, hypoxia, and hypotension (64, 65). Because both acetoacetate and β-hydroxybutyrate freely cross the cell membrane, abnormalities of this ratio can easily be detected by arterial blood measurements.

Patients with severe hepatic failure tend to die when the arterial blood ketone body ratio decreases markedly, accompanied with a marked decrease in the hepatic energy charge (66–68). Although the regulating mechanism remains to be clarified, ketogenic adaptation in the liver may play an important role in restoring mitochondrial impairment (69). As the arterial ketone body ratio decreases, hepatic fatty acid oxidation is enhanced with a tendency toward increased ketogenesis. However, while ketogenic adaptation progresses dramatically, as evidenced by a further decrease in the arterial ketone body ratio, prolongation of that state reduces the ketone body ratio, because even fatty acids can no longer be utilized in the terminal state.

The relation of arterial ketone body ratio to clinical status has been examined in a prospective study of 55 patients undergoing major abdominal surgery (70). Ozawa et al. (70) found that patients whose ketone body ratio never fell below 0.7 had an uncomplicated postoperative recovery. Of patients in whom the ratio fell to <0.25, nearly all went into pulmonary, hepatic, renal, cerebral, and cardiac failure. In patients with transient organ failure before recovery, the ketone body ratio fell and rose accordingly.

Plasma amino acids were also measured in several of these patients; the blood ketone body ratio correlated negatively with alanine, proline, phenylalanine, and tyrosine. Ozawa et al. suggest that when the ketone body ratio was reduced, entry of these amino acids into the Krebs cycle was inhibited, leading to an accumulation in the
blood. Hepatic metabolism played a key role in this study, with 85% of their patients having hepatobiliary disorders; the remaining 15% belonged to the mildest category of ketone body ratio abnormality.

Recently, a study of surgical stress in a random selection of 60 patients undergoing elective laparotomy was performed, utilizing arterial ketone body ratio and a hepatic stress score (total area below the ketone body ratio of 0.7) (71). This measure of hepatic functional capacity indicated a causative relationship between suppression of hepatic energy metabolism during the operation and enhanced postoperative catabolic response. Results suggest that total surgical stress in major laparotomy can be quantified and evaluated through the magnitude of decrease in hepatic mitochondrial redox potential.

Each organ is functionally integrated with all others by an extensive metabolic regulation system. The exchange of energy and substrates between organs is crucial for inducing the steady-state favorable to maintaining life. The liver plays a central role in this homeostasis and is being increasingly recognized as a critical organ in the pathogenesis of MOFS. Evidence now suggests that the functional reserve of the liver can be noninvasively estimated by determining the ketone body ratio, which may function as a systemic indicator of metabolic derangement.

Cytokines

Recent advances in molecular biology have allowed the recognition of a multitude of factors involved in the regulation of cell growth and metabolic functions. New developments in molecular techniques have made possible the purification, characterization, and eventual production by recombinant technology of many of these substances.

Cytokines, soluble factors that can modify the response and (or) growth pattern of other cells (72), are produced by a variety of cells, including blood monocytes, tissue macrophages, keratinocytes, and endothelial cells (73). Cytokines resemble hormones in their mechanisms of action, but are released and active at such a small amount that they usually are not detectable in the systemic circulation (72). These mediators, such as tumor necrosis factor (TNF; also known as " cachectin") and the interleukins (ILs), play a determining role in the regulation of cellular processes, which include immune recognition, differentiation, and cell proliferation (74-76). Early studies on the metabolic effects of cytokines demonstrated that bacterial endotoxins were potent stimulators of IL-1 and TNF (77-79). Later studies, using recombinant versions of IL-1-α and -β and TNF-α and -β, revealed that these cytokines could stimulate each other's production (80, 81) and act synergistically (82, 83).

TNF, now termed TNF-α, was originally identified as a product of macrophage stimulation by endotoxin (84). The primary effect of TNF on tumor tissue is hemorrhagic necrosis; it also increases the permeability of vessel walls. In vitro analysis has revealed potent inhibitory effects on erythropoietin/myeloid proliferation, activation of neutrophils, and intrinsic antiviral activity (72). TNF has been correlated with increased muscle energy production by promoting "futile cycles," i.e., energy that cannot be converted into muscle contraction, and results in loss of lean body mass (85).

IL-1, which starts T-cell proliferation and activates T-cell production of gamma interferon and IL-2, has been identified as a potent mediator of inflammation, causing fever, release of acute-phase proteins, cartilage breakdown, and activation of neutrophils, macrophages, and T lymphocytes (86). IL-2 is secreted by activated T helper cells in response to antigen presentation. IL-2 is taken up by activated T killer cells, which thereby become fully activated, capable of destroying target cells such as cancer or viral infected cells (87).

Although TNF-α and ILs affect many cell functions, their molecular mechanisms of action are not understood. Most cytokines must bind to a specific receptor on their target cell to produce an intracellular signal ("second message"), resulting in a cellular response (72, 88). A number of second messengers such as cAMP and protein kinase C have been described (89). It has been hypothesized that each cytokine has a cascade system that consists of several messengers and results in delivery of its own individual signal to the cell. In addition, this system may modulate receptor expression to other substances, thereby altering cellular response to these factors (72). The end result of this process is the modulation of gene expression, which may occur at the transcription of mRNA from DNA or at the translation of mRNA into protein. The signal may be stimulatory or inhibitory (72, 90).

Normal physiological production of cytokines results in stimulation and enhancement of immune system activity. However, greater cytokine production, particularly of TNF-α, is toxic to the host (91). Recent studies demonstrate that an exaggerated TNF response often leads to hemodynamic collapse, shock, and death (92). TNF-α has been implicated as a mediator that is released in response to lipopolysaccharide (endotoxin) stimulation and is capable of initiating multiple organ injury (93, 94).

It has been suggested that the deleterious effect associated with TNF in human septic shock occurs in cooperation with other factors. Prostaglandins and leukotrienes have been implicated in the shock-like hypothermic effects of TNF-α when given at doses in the pathological range (95). A recent study demonstrated that during the initial phase of meningococcal septic shock IL-6 and IL-1 are released into serum, and concurrently are found with TNF-α and lipopolysaccharide in the systemic circulation. High concentrations of IL-6 in serum were associated with fatal outcome. IL-1 was exclusively detected in patients who had high concentrations in serum of IL-6, TNF-α, and lipopolysaccharide, and had a rapid fatal outcome (96).

Complex interactions between nutrition, infections, and diseases that have an inflammatory component are currently an area of intense investigation. Cytokines are well recognized to play an essential role in these interactions. Numerous studies show that nutritional factors affect the tissue targets of cytokine action and the cells of the immune system from which cytokines are produced. Therefore, nutrients and nutritional status not only may influence the ability of the consumed and traumatized individuals to produce cytokines, but also may affect subsequent cytokine actions (97). In vitro studies with IL-1, TNF-α, and IL-6 suggest that cytokines are central to the stimulation of acute-phase protein synthesis and alterations in the serum protein profile during an inflammatory response (88, 99).

Evidence of malnutrition is the common feature in decreased cytokine production in studies of hospitalized patients. Improvement in the ability to produce cytokines has been noted in some patients, which was related to protein intake; patients who showed no improvement had a high mortality rate (100). We cannot determine from the data in these studies whether IL-1, IL-6, or TNF-α production was
reduced specifically, because the bioassays used did not differentiate between these cytokines (97).

Deficiencies of trace elements and vitamins are commonly found in malnourished populations. However, few studies have investigated the effects of individual deficiencies on cytokine production or actions (97). Peritoneal macrophages from severely iron-deficient rats were noted to have impaired ability to produce cytokines, whereas treating anemic rheumatoid patients with iron exacerbated inflammatory symptoms (101, 102). Reduction in cytokine production was noted in a recent study in which mice receiving a copper-deficient diet showed only 64% of the normal increase in spleen cells when exposed to E. coli lipopolysaccharide (103).

Of the vitamins, vitamin A is particularly important in maintaining adequate cell-mediated immunity. A study by Moriguchi and Werner (104) showed that stimulated peritoneal macrophages of mice given 16 times the normal intake of vitamin A showed a twofold increase in IL-1 production. It is unclear whether the reduced resistance to infection found during vitamin A deficiency, or the antitumor effect of high doses of retinoids, is significant.

Future Directions

Further investigation examining the precise way in which specific nutrients exert effects on production and action of cytokines is necessary. Insufficiency of nutrients may prevent cytokines from optimizing beneficial effects in the recovery process. A more complete understanding of nutrient-cytokine and cytokine-cytokine interactions is also important in situations where cytokines are being used therapeutically, such as in cancer treatment. However, interpretation of study results must be approached with caution. The significance of cytokine concentrations in plasma is questionable, given growing evidence that cytokines are rapidly cleared from the circulation (97). The likelihood that many cytokines are produced locally and act locally makes development of reliable methodology to observe these phenomena a priority.

In addition, the synergistic interaction of cytokines, particularly in inflammatory diseases, suggests that caution should be exercised in interpreting the activity of a single cytokine. The use of monoclonal antibodies for cytokines will help clarify mechanisms of action and enable more discerning evaluation of data from these studies. Their potential utility in predicting the development of shock states and in monitoring and predicting outcome also remains to be evaluated.

Immune Function

Suboptimal nutrition results in profound compromise of the immune response (105, 106). Negative nitrogen balance, weight loss, and substantial immune dysfunction are common consequences of major surgery, various disease states, and trauma. While positive nitrogen balance and weight gain can be accomplished through perioperative enteral and parenteral nutrition, the general effects of nutritional support on immune function are unclear (107, 108). Nutritional support can reduce the morbidity and mortality associated with starvation. However, it has not altered the course of disease, including hypermetabolism and MOFS in the critically ill patient (109).

Substrates in the nutrition regimen apparently affect nutritional and nonnutritional markers. Recent data suggest that nutrients such as arginine (27), polyunsaturated fatty acids (110), and purines/pyrimidines (111) may profoundly affect lymphocyte function, even under conditions of infection or major general surgery (112).

Arginine

Arginine is a nitrogen-dense amino acid having multiple biologically important properties. A semi-essential amino acid, it is required for growth and for recovery in post-traumatic states (113, 114). Arginine is a potent secretagogue, influencing many endocrine glands, and appears to be a modulator of the immune system (114–116). In humans, arginine supplementation increases blastogenesis of peripheral blood lymphocytes in response to certain mitogens (117). Arginine prevents or diminishes the reduction in blastogenesis in immunosuppressed and postoperative cancer patients (118). Improved mitogenesis has been observed in patients who are positive for Human Immunodeficiency Virus, although without apparent effect on peripheral lymphocyte ratios (119).

Ribonucleic Acid

Investigations over the past several years have revealed the vital role dietary nucleotides play in the maintenance of T-lymphocyte-mediated immunity. Nucleotides are precursors of DNA and RNA, which are necessary for protein synthesis and cell mitosis. They play major roles in almost all biochemical processes.

The liver has been suggested to be a major source of preformed purines and pyrimidines for other tissues, but comparative contributions are not well documented. Bases or nucleosides are re-utilized through salvage pathways and are degraded when not required. Both dietary pyrimidines and purines decrease de novo pyrimidine biosynthesis in humans (120), and dietary uracil contributes to cellular pyrimidine pools (121).

Removal of dietary nucleotides results in suppression of cellular immune responses (122, 123), which, in part, is a result of a block in lymphocyte maturation (124). Provision of uracil, unlike adenine, has demonstrated restoration of delayed-type hypersensitivity response to various foreign antigens in mice (125, 126). Dietary nucleotides may also be effective in macrophage activation of the T helper/inducer population (127). In experimental settings, uracil has been demonstrated to reverse the immunosuppression associated with blood transfusion.

Omega-3 Fatty Acids

Lipid intake can exert metabolic effects by altering the fatty acid composition of cell membranes (128). The lipid composition of membranes alters membrane fluidity and the generation of second messengers, and thus the pattern of prostaglandin and leukotriene synthesis is changed by alterations in lipid substrate (129). Generally, the eicosapentaenoic acid (20:5 n-3) products are less inflammatory than those of linoleic acid (18:2 n-6) (130). A relative excess of linoleic acid substrate stimulates production of prostaglandin PGE2, which decreases the ability of cytokines to stimulate IL-2 synthesis by endothelial cells (131).

The release of diencephalic eicosanoids and TNF, and IL-1 released by Kupffer cells, are directly related to the amount of n-6 PUFA in the cell membrane. The incorporation of the PUFAs into cell membranes can occur within hours of ingestion and become stabilized within two to four days. In animal models, incorporation of n-3 PUFA into cell membranes of the hepatic macrophages, with a reduction in the
n-6 content, was associated with a reduction in mortality in the presence of cecal ligation and puncture peritonitis. In models of allogenic stimulation, such as the popliteal lymph node assay system, n-3 PUFA was associated with a return in the proliferative response in a magnitude equivalent to RNA.

The interrelationship between altered immune function and nutritional status has been difficult to define. Many factors other than nutritional status may result in an altered immune response of the hospitalized patient. In addition, existing tests of immune function tend to lack both sensitivity and specificity, further confounding delineation of the relationship between nutritional status and immune function.

Study Methods

In the past, immune-function studies have been performed on isolated individual cell populations by time-consuming manual methods. Results of these assays generally reflect the average function of an entire population of cells tested. Advances in monoclonal antibody technology and the quantitative multianalyte analysis capability of flow cytometry make possible routine determination of leukocyte subpopulations in blood or from cell cultures (132).

The most often-analyzed marker of lymphocyte function is that of lymphocyte blast transformation in response to various mitogens such as phytohemagglutinin, concanavalin A, or pokeweed mitogen (133). The proliferation of responsive lymphocytes can be measured by the incorporation of a radiolabeled nucleotide in newly synthesized DNA or by staining the total DNA content of cells with a fluorescent DNA-specific dye and quantifying the fluorescence distribution of a flow cytometer. Flow-cytometric analysis of lymphocyte transformation allows the precise determination of the exact cell-cycle distribution of responding cells at any time during the culture period (132).

Recently, a new methodology has been described to quantify the release of important cell-surface markers into a plasma or culture supernate. Cells actively participating in an immune response will shed physiologically important markers such as IL-2 receptors and CD8 molecules. The concentrations of these markers found in the circulation have been shown to be important predictors of immune response capability in several models. The assays are enzyme-linked immunosorbent assay procedures and are easily performed (133).

Tests for neutrophil and monocyte function are also readily available. Flow cytometry again provides a quantitative assessment of such functional measures as phagocytosis and oxidative respiratory burst activity. These markers are valuable in evaluating the functional potential of the cells in the first line of defense against bacterial infection (132, 133).

Several soluble products have recently been identified as important components in either maintaining or quantifying the immune status of patients. Mediators such as the lymphokines IL-1 and IL-2 are required to mount immune responses to antigenic challenge. Other components such as cell-surface antigens, e.g., IL-2 receptors and CD8, have been shown to be shed from immunologically active cells. Quantification of these shed cell-surface antigens in plasma or in cell culture supernate can serve as a sensitive indicator of immune activation (133).

The ICU patients under discussion in the paper are all significantly immunosuppressed. Recent studies indicate that nutrients such as arginine, RNA, and n-3 PUFA, all targeted to improve T-lymphocyte function and to alter macrophage activity, can significantly improve the in vitro tests of lymphocyte function that evaluate proliferative responses to stimulation by specific and nonspecific antigens. The utility of these nutrients in altering clinical outcome for patients is also currently in clinical testing (113).

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